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VEGF + FGF2
103 Ref.?

Vascular Endothelial Growth Factor Mediates Angiogenic Activity during the Proliferative Phase of Wound Healing

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Angiogenesis is an essential component of normal wound repair, yet the primary mediators of wound angiogenesis have not been well described. The current study characterizes the contribution of vascular endothelial cell growth factor (VEGF) to the angiogenic environment of human surgical wounds. Surgical wound fluid samples ($n = 70$) were collected daily for up to 7 postoperative days (POD) from 14 patients undergoing mastectomy or neck dissection. VEGF levels in surgical wound fluid were lowest on POD 0, approximating values of serum, but increased steadily through POD 7. An opposite pattern was noted for basic fibroblast growth factor-2. Fibroblast growth factor-2, which has been previously described as a wound angiogenic factor, exhibited highest levels at POD 0, declining to near serum levels by POD 3. Surgical wound fluid from all time points stimulated marked endothelial cell chemotaxis and induced a brisk neovascular response in the rat corneal micropocket angiogenesis assay. Antibody neutralization of VEGF did not affect the *in vitro* chemotactic or the *in vivo* angiogenic activity early wound samples (POD 0). In contrast, VEGF neutralization significantly attenuated both chemotactic activity (mean decrease 76 ± 13%, $P < 0.01$) and angiogenic activity (5 of 5 samples affected) of later wound samples (POD 3 and 6). The results suggest a model of wound angiogenesis in which an initial angiogenic stimulus is supplied by fibroblast growth factor-2, followed by a subsequent and more prolonged angiogenic stimulus mediated by VEGF. ***(Am J Pathol 1998, 152:1445–1452)***

Normal wound repair includes a vigorous angiogenic response that delivers nutrients and inflammatory cells to injured tissue. This angiogenic response facilitates the removal of debris and assists in the development of a granulation tissue framework for wound closure.[1] The mediators of wound angiogenesis are thought to include numerous soluble factors that have been identified in various wound models.[2,3] Both angiogenic agonists and antagonists have been identified at various times during repair,[4,5] suggesting that the net angiogenic stimulus may change as the balance of factors alternatively favors either vessel growth or regression.[6,7] Despite a large body of literature describing the production of angiogenic growth factors in wounds, few previous studies have attempted to determine which factors are critical mediators in healing wounds or to understand their temporal relationship to one another.

To better understand the mechanism of wound angiogenesis, recent efforts in our laboratory have been directed at identifying the soluble proangiogenic factors that are important to normal wound healing. As part of this undertaking, the angiogenic profile of fluid collected from human surgical wound drains placed at the time of various operations has been characterized.[8] These studies are based on the premise that wound fluid would be generally representative of the growth environment of the wound, as it would be expected to contain soluble proangiogenic growth factors at levels similar to that of the wound bed itself. While the proliferative capacity of wound fluid has been previously reported, studies of the net angiogenic stimulus in this fluid[9] and of the specific mediators responsible for this stimulus are incomplete.

In a previous report from our laboratory, surgical wound fluid (SWF) collected within just a few hours of operation was found to be potently angiogenic. This fluid stimulates endothelial cell migration and proliferation *in vitro* and new vessel formation in the *in vivo* rat corneal assay.[8] The angiogenic activity of early wound fluid was

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found to be largely because of basic fibroblast growth factor (FGF-2). FGF-2 levels in SWF peaked immediately after surgery and then fell to near-serum levels by the second postoperative day. Thus FGF-2, stored either within cells or sequestered in the extracellular matrix, is released shortly after tissue injury. This quick release of FGF-2 appears to function to initiate wound angiogenesis. The current study was designed to identify the primary angiogenic mediators in later wounds during the proliferative phase. To accomplish this, wound fluid was collected from surgical wounds for up to 7 days postoperatively. This fluid was used to assess the role of particular growth factors as critical angiogenic mediators in human wounds. Among the many mediators in the later wound fluid, our studies quickly focused on vascular endothelial cell growth factor (VEGF) for two specific reasons. First, like FGF-2, VEGF is a potent direct angiogenic factor that stimulates endothelial cell migration and activation *in vitro* and angiogenesis *in vivo*.[10,11] Secondly, an up-regulation of VEGF production in wound repair has been demonstrated in keratinocytes in skin wounds in rat, guinea pig, and mouse models.[12,13] The current study describes the contribution of VEGF to the angiogenic activity within wounds and establishes an important role for VEGF as a predominate angiogenic mediator in human surgical wounds.

Materials and Methods

Patients

Surgical wound fluid was collected from 14 patients undergoing modified radical mastectomy (n = 8) or neck dissection (n = 6). This group consisted of 11 females and 3 males and averaged 60 ± 14 years of age. A total of 70 samples were collected between postoperative days 0 and 7. Most samples collected beyond 4 days were from neck dissections because of the tendency to remove drains earlier from mastectomy sites as compared with neck procedures. No wounds were complicated by hematoma formation, infection, or flap necrosis. Breast biopsy specimens were obtained from 4 patients undergoing mastectomy 7, 8, 10, and 22 days after initial biopsy. No patient had received heparin, corticosteroids, or preoperative chemotherapy or radiation therapy before or during the time of sample collection. No patients had complicating disease processes that might affect wound healing, such as diabetes mellitus.

Wound Fluid Samples

Surgical wound fluid was collected from closed suction drains of patients undergoing either modified radical mastectomy or neck dissections. Neck dissections were usually performed in combination with a pharyngectomy or laryngectomy. On Postoperative day (POD) 0, fluid was collected within 6 hours of surgery. Subsequent samples were obtained daily until patients were discharged or drains were removed. Occasionally patients were unavailable during the collection period and a daily sample was not obtained. Fluid samples were processed immediately after collection by centrifuging at 1300 × g for 10 minutes and then freezing at −70°C. Normal serum was obtained from volunteers and was processed as described for SWF samples. When subsets of patient samples were used in additional assays, samples were selected randomly. The average total protein content of the wound fluids was 17 ± 4 mg/ml.

VEGF and FGF-2 Enzyme-Linked Immunosorbent Assay

The level of VEGF in SWF samples was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) that detects the soluble isoforms (VEGF 121 and VEGF 165) of human VEGF (R & D Systems, Minneapolis, MN). To directly compare the profile of VEGF with that of FGF-2, which is known to contribute to the angiogenic activity of SWF, we also measured FGF-2 levels using an ELISA directed at recombinant human $FGF\text{-}2_{145}$ (R & D Systems). Each sample was tested in duplicate, and results were averaged. Samples from each POD are presented as a group mean ± SD. Results were analyzed for significance by a one-way analysis of variance followed by Tukey-Kramer multiple comparisons test.

Endothelial Cell Migration

Endothelial cell chemotactic activity in response to SWF was measured as previously described.[14] Human dermal microvascular endothelial cells and growth medium were obtained from Cell Systems (Kirkland, WA). Endothelial cells in suspension were placed in the bottom wells of a 48-well blind well microchemotaxis chamber (Neuroprobe, Cabin John, MD) and overlaid with a gelatinized 5-μm pore membrane. The top portion of the chamber was secured in place, and 50 μl of test or control medium was dispensed into the top wells. The test medium contained recombinant VEGF or FGF-2 in amounts ranging from 1 to 50 ng/ml or wound fluid samples containing 1 μg of total protein. Chambers were inverted and incubated for 2 hours at 37°C, after which the membranes were stained with Wright's Giemsa stain (Leukostat; Fisher Scientific, Chicago, IL). The number of endothelial cells that had migrated to the opposite surface of the membrane was counted in 10 random high-power fields. Each sample was tested in triplicate. Results are expressed as a mean number of endothelial cells migrated per 10 high-powered fields ± SD. Chemotactic activity of individual samples was compared with control medium using a Student's t-test and Bonferroni postcomparison testing. P values less than 0.05 were considered significant.

Bioassay for Angiogenic Activity

Assays of angiogenic activity were performed in the avascular cornea of the rat eye as previously de-

scribed.[15] Briefly, wound fluids containing approximately 5 μg of total protein were incorporated 1:1 into noninflammatory Hydron polymer (Interferon Sciences, New Brunswick, NJ), and 5-μl pellets were implanted into the corneal stroma 1 to 1.5 mm from the limbus. Corneas were examined daily with a stereomicroscope to monitor capillary growth by blinded observers. Responses showing sustained ingrowth of a brush-like network of capillary sprouts were judged to be positive, whereas responses showing no growth or only occasional sprouts were considered negative. Individual wound fluid samples were tested in multiple corneas and were considered angiogenic if more than 50% of the corneas showed a positive angiogenic response. Positive controls included VEGF (25 ng) and FGF-2 (50 ng). Dulbecco's modified Eagle's medium was used as a negative control. Five to 7 days after implantation some rats underwent intra-arterial perfusion with colloidal carbon to provide a permanent record of individual responses. Corneas were then excised, fixed in 2% glutaraldehyde-2.5% paraformaldehyde, flattened, and photographed.

Neutralization of VEGF

To determine the contribution of VEGF to the angiogenic activity of SWF, neutralizing antibody to VEGF was combined with SWF before addition to *in vitro* and *in vivo* assays. Antibody directed against recombinant human $VEGF_{165}$ was obtained from R & D Systems. 250 ng of anti-VEGF antibody was incubated with SWF or 25 ng of human recombinant VEGF (R & D Systems) for 30 minutes at 37°C before testing.

Immunohistochemistry

Samples of healing wounds were obtained by excising old biopsy sites from the pathological specimens of four patients undergoing mastectomy. Each patient had undergone an excisional biopsy before mastectomy, and each biopsy specimen had been determined to have tumor-free margins. Full thickness skin and subcutaneous tissue samples were embedded in OCT and sectioned perpendicular to the healing incision line. Immunostaining was performed using an avidin-biotin technique (Vector Laboratories, Burlingame, CA). Slides were fixed in cold acetone for 20 minutes followed by treatment with 3% peroxide in 0.1 mol/L Tris for 5 minutes to block endogenous peroxidase activity. All subsequent incubations were performed at 37°C in a humidified chamber unless otherwise noted. Tissue were pretreated with 3% horse serum in phosphate-buffered saline (PBS) for 30 minutes, incubated with anti-human VEGF mAb (gift of Dr. Napoleone Ferrara, Genentech, South San Francisco, CA), anti-human Leu-M5 (CD11c) (Becton Dickinson, San Jose, CA), or mouse IgG1 control for 45 minutes, and washed twice in PBS. Slides were then incubated with a 1:200 dilution of anti-mouse biotinylated antibody in PBS containing 3% horse serum, washed twice with PBS, incubated with avidin-conjugated horseradish peroxidase complex at room temperature for 20 minutes, and washed twice with PBS. Slides were then incubated with diaminobenzidine tetrahydrochloride substrate for 5 minutes at room temperature, rinsed in tap water, counterstained with Harris' hematoxylin, and dipped in saturated lithium carbonate solution for bluing. Serial sections were examined to assess the cell types expressing antigenic VEGF.

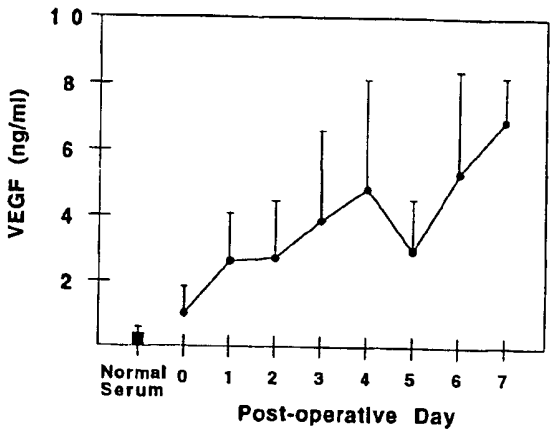

Figure 1. VEGF levels in surgical drain fluid. Surgical drain fluid was collected as described in the text and VEGF levels determined by ELISA. VEGF levels were lowest on postoperative day 0 and rose steadily to peak on postoperative day 7. The sample sizes are as follows: day 0, $n = 8$; day 1, $n = 10$; day 2, $n = 10$; day 3, $n = 8$; day 4, $n = 8$; day 5, $n = 5$; day 6, $n = 6$; and day 7, $n = 2$. On days 4, 6, and 7, fluids contained significantly more VEGF than fluids on day 0 ($P < 0.05$).

Results

VEGF Levels in SWF

The level of VEGF in 57 SWF samples was measured by ELISA (Figure 1). Normal serum contained on average 0.4 ± 0.2 ng/ml VEGF. SWF levels on POD 0 were slightly higher than serum averaging 1.0 ± 1.0 ng/ml. VEGF levels increased to 3.9 ± 2.7 ng/ml on POD 3 and were highest by POD 7 at 6.9 ± 1.3 ng/ml. This steady rise in VEGF levels over time was similar in both mastectomy and neck wounds and was unchanged when samples were corrected for total protein content. Although tumors have been shown to produce VEGF, residual tumor is unlikely to be the source of the VEGF for two reasons. First, the margins on all sites were determined to be free of tumor by a surgical pathologist. Second, the VEGF did not appear in the fluid until several days after surgery, suggesting that new synthesis was initiated at the site of injury.

FGF-2 Levels in SWF

To directly compare the profile of VEGF and FGF-2 in surgical wounds, the level of FGF-2 in 25 SWF samples was also measured. While VEGF is lowest on POD 0 and then rises over several days, FGF-2 levels were highest on POD 0 at 765 ± 436 pg/ml and then fell rapidly over the next 1 to 2 days (Figure 2). Both mastectomy and

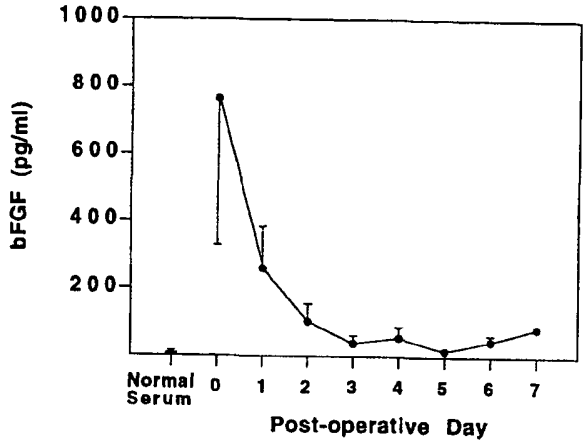

Figure 2. FGF-2 levels in surgical drain fluid. Surgical drain fluid FGF-2 levels were measured by ELISA. In contrast to VEGF, the level of FGF-2 in surgical drain fluid is highest on postoperative day 0 and then falls quickly to near serum levels by postoperative day 2. The sample sizes are as follows: day 0, $n = 8$; day 1, $n = 8$; day 2, $n = 7$; day 3, $n = 7$; day 4, $n = 5$; day 5, $n = 4$; day 6, $n = 4$; and day 7, $n = 1$. On day 0, fluid contained significantly more FGF-2 than all other days ($P < 0.001$).

neck dissection wounds demonstrated similar FGF-2 profiles. These results are similar to our previous report.[8]

VEGF-Mediated Activity in Surgical Wound Fluid — Endothelial Cell Migration

The endothelial cell chemotactic activity of seven random SWF samples from postoperative days 0, 3, and 6 was determined using a modified Boyden chamber. SWF samples from all 3 days stimulated marked endothelial cell chemotaxis compared with basal media (Figure 3; $P < 0.01$ for all samples). The addition of anti-VEGF neutralizing antibody did not affect the chemotactic activity of two SWF samples from POD 0 (average decrease in activity 12 ± 6%). Anti-VEGF antibody lowered the chemotactic activity of five samples from POD 3 and 6 by an average of 76 ± 13% (Figure 3). VEGF-mediated endothelial cell chemotactic activity therefore parallels VEGF protein levels in SWF. While VEGF appears to be the primary chemotactic agent by the third postoperative day, it does not contribute significantly to the activity of POD 0 SWF.

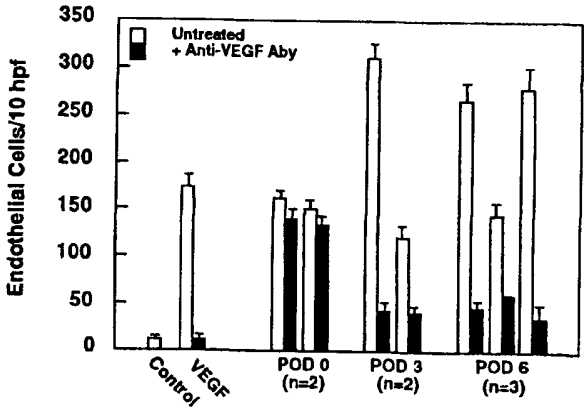

Figure 3. VEGF-mediated endothelial cell chemotactic activity of surgical drain fluid. Fluid from surgical wounds was tested for endothelial cell chemotactic activity in a modified Boyden chamber as described in the text. Random samples from POD 0 through 6 consistently demonstrated marked chemotactic activity compared with control media ($P < 0.01$ for each). Anti-VEGF antibody markedly diminished the chemotactic activity of recombinant VEGF (25 ng) and of five samples from POD 3 and 6 but did not alter the activity of two samples from POD 0.

Table 1. Effect of Anti-VEGF Antibody on Angiogenic Activity of Wound Fluid

Surgical wound fluid sample	Rat corneal neovascularization: proportion of positive responses (%)	
	No treatment	+Anti-VEGF antibody
Postoperative day 0		
Mastectomy 1	2 of 2 (100)	2 of 2 (100)
Neck dissection 2	2 of 2 (100)	2 of 2 (100)
Postoperative day 3		
Mastectomy 3	3 of 3 (100)	0 of 3 (0)
Mastectomy 4	2 of 2 (100)	1 of 3 (33)
Mastectomy 5	3 of 3 (100)	nd
Mastectomy 6	2 of 2 (100)	nd
Postoperative day 4		
Mastectomy 7	3 of 3 (100)	nd
Postoperative day 6		
Neck dissection 2	3 of 3 (100)	0 of 3 (0)
Neck dissection 3	3 of 3 (100)	0 of 2 (0)
Mastectomy 4	2 of 2 (100)	1 of 4 (25)
Controls		
DMEM*	0 of 3 (0)	0 of 3 (0)
VEGF (25 ng)	3 of 3 (100)	1 of 4 (25)

*DMEM, Dulbecco's modified Eagle's medium; nd, not done.

VEGF-Mediated Activity in Surgical Wound Fluid — In Vivo Angiogenic Activity

SWF samples from POD 0, 1, 3, and 6 stimulated angiogenesis in the avascular rat cornea assay (Table 1, Figure 4A). The neovascular response was uniform across different postoperative days, generally occurring rapidly and without an excessive inflammatory response. Similar to the results reported above, anti-VEGF antibody did not affect the *in vivo* angiogenic response to two POD 0 samples but significantly reduced the angiogenic activity of five of five SWF samples from POD 3 and 6 (Table 1, Figure 4B). VEGF therefore mediates in large part the chemotactic and angiogenic activity of SWF from postoperative days 3 and 6 but not of fluid from postoperative day 0.

Source of VEGF in Wound Biopsies

Healing breast biopsy sites were analyzed with immunohistochemical staining to identify the source of wound VEGF. Four samples were obtained from 7 to 22 days after initial biopsy. Compared with control skin, staining for VEGF was substantially increased at wound sites. In wounds, VEGF staining was mainly localized to fibroblasts and macrophages (Figure 5). Normal skin from adjacent uninjured areas of the breast demonstrated minimal staining for VEGF. This staining was localized to

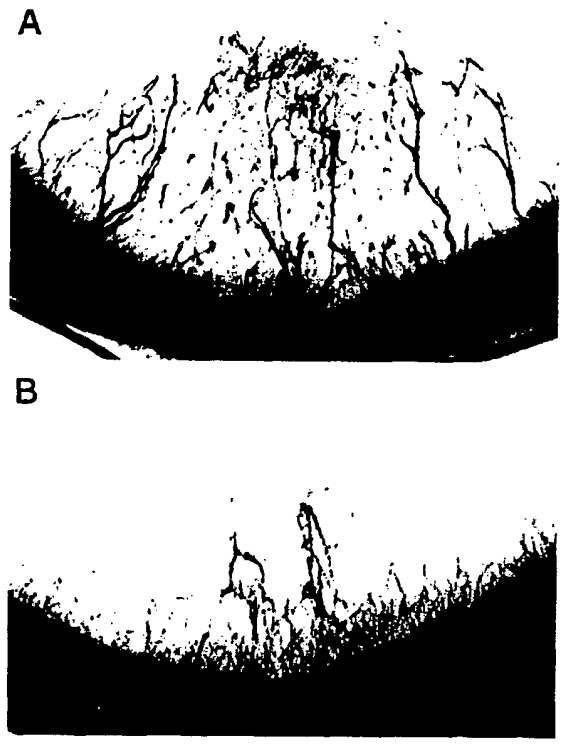

Figure 4. Rat corneal neovascularization in response to mastectomy drain fluid from postoperative day 6. Dense new vessel ingrowth from the corneal limbus toward a pellet containing drain fluid is demonstrated (A). Addition of neutralizing anti-VEGF antibody (B) dramatically diminished the response to wound fluid.

fibroblasts. Normal skin macrophages were generally negative for VEGF. Neutrophils, lymphocytes, endothelial cells, and mast cells were generally immunonegative for VEGF in both wounds and normal skin.

Discussion

VEGF has been associated with angiogenesis in numerous pathological situations, including tumor growth, proliferative retinopathy, and rheumatoid arthritis.[16-18] In particular, a great amount of interest has centered on the role of VEGF in the growth and metastasis of several tumor types and on the potential role of inhibitors of VEGF in antitumor therapy.[16,19] This interest was borne in part from the original description of VEGF as a potent vascular permeability factor, a property that was thought to contribute to tumor metastases.[20,21]

Increased vascular permeability has since been shown to occur during the early phases of wound repair, theoretically allowing deposition of the fibrin-rich matrix necessary for cellular migration.[22,23] The identification of increased vascular permeability concomitant with increased VEGF production in skin wounds provided evidence for a role for VEGF in wound repair.[12] However, VEGF-mediated angiogenic activity in wounds had remained speculative.

Our results provide two lines of evidence to support a central role for VEGF in mediating angiogenesis in surgical wounds. First, VEGF is produced or released locally in wounds during a time when new vessel growth is initiated and maintained. Second, neutralization of VEGF greatly decreases the angiogenic and endothelial cell chemotactic activity of surgical wound fluid. In the current study, VEGF levels in wound fluid rose steadily through the first week after injury. New vessel formation in healing wounds follows a similar time course with newly formed vessels first evident 2 to 3 days after injury with maximal evidence at 1 to 2 weeks.[24] By postoperative day 3, VEGF appears to be the primary angiogenic mediator in SWF as addition of neutralizing antibody eliminated most of the chemotactic and angiogenic activity of all samples tested. The current study does not examine how wound proteases may influence the levels of bioactive FGF-2 and VEGF. Wound fluid has been described to contain high levels of proteases, such as metalloproteinases.[25] Although we did not examine the levels of protease activity in our samples, our results suggest that the protease activity in wound fluid is not sufficient to eliminate bioactive growth factors such as FGF-2 and VEGF.

To our knowledge this report is the first direct evidence of VEGF-mediated angiogenic activity in human surgical wounds. VEGF may contribute to the angiogenic stimulus in wounds either by direct effects on proliferating and migrating endothelial cells or indirectly by effecting persistent vascular permeability at the level of existing microvessels. Mitigation of endothelial cell chemotactic activity of SWF by anti-VEGF antibody suggests that at least part of the stimulus provided by VEGF in the surgical wound is direct.

Our results demonstrate that the primary sources of surgical wound VEGF are the fibroblast and the macrophage. Both cell types have been described to produce VEGF *in vitro*.[26,27] Previous studies by Brown et al[12] also identified macrophage-like cells as a source of VEGF in mouse skin wounds but found keratinocytes to be the major source of VEGF. Keratinocytes in healing breast biopsy sites did not stain for VEGF in our hands. This discrepancy between our results and those of Brown et al probably reflects the inherent differences between skin and deep surgical wounds. Keratinocytes would not be expected to contribute much to VEGF levels in deep surgical wounds because of the relatively small area of epidermal injury in these wounds.

While VEGF appears to be a primary angiogenic mediator in surgical wounds by several days postoperatively, it does not appear to participate in the initiation phase of angiogenesis. SWF obtained immediately after injury (POD 0) stimulates angiogenesis and chemotaxis but contains low levels of VEGF and is not affected by neutralizing anti-VEGF antibody. We have previously reported that FGF-2 accounts for a large part of the immediate angiogenic stimulus in SWF based on similar neutralization experiments.[8] FGF-2 levels peak within hours of injury and fall as VEGF levels start to rise. This pattern suggests that FGF-2 and VEGF act in concert to provide a sustained angiogenic stimulus in surgical wounds, as

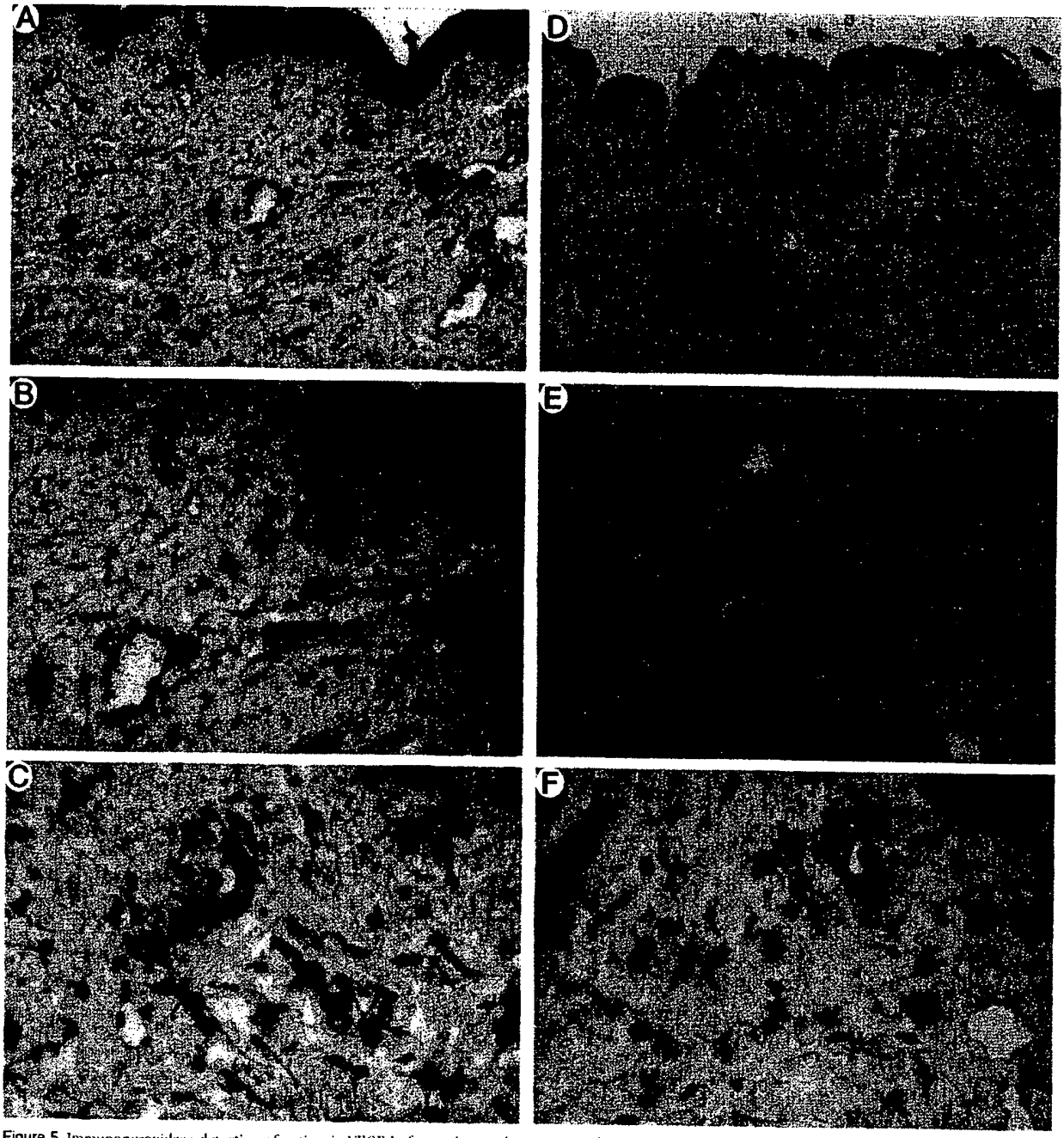

Figure 5. Immunoperoxidase detection of antigenic VEGF in frozen human breast tissue. **A:** Breast biopsy site showing strong staining of both fibroblasts and macrophages. Magnification, ×264. **B:** Higher power image of **A**, showing fibroblast staining. Magnification, ×528. **C** Higher power image of **A**, showing macrophage staining (**arrows**). Magnification, ×1056. **D:** Normal breast skin showing minimal fibroblast staining. Magnification, ×264. **E:** Higher power image of tissue in **D**, showing minimal fibroblast staining. Magnification, ×528. **F:** Immunoperoxidase detection of Leu-M5 (CD11c), a macrophage marker, in a serial section to the tissue in **C**, showing staining in a similar pattern as the VEGF staining. Magnification, ×1056.

the angiogenic stimulus initiated by FGF-2 is subsequently maintained by VEGF.

The FGF-2 and VEGF-mediated angiogenic responses in wounds may represent two components of an angiogenic cascade. FGF-2 is well suited to initiate angiogenesis because it is available immediately after injury and because it affects numerous cell types. Preformed FGF-2 that is sequestered in uninjured tissue is likely released at the time of surgery either by cell damage or by enzymes such as thrombin.[28-31] Because multiple cell types carry the FGF receptor, soluble FGF-2 stimulates multiple early wound events, including the proliferation and migration of both endothelial cells and fibroblasts.[32] In this way angiogenesis and collagen deposition may be initiated while synthesis of mediators such as VEGF is beginning. Unlike the broadly distributed FGF-receptors, VEGF-receptors are almost exclusive to endothelium.[33] Transition to a predominantly VEGF-mediated angiogenic stimulus

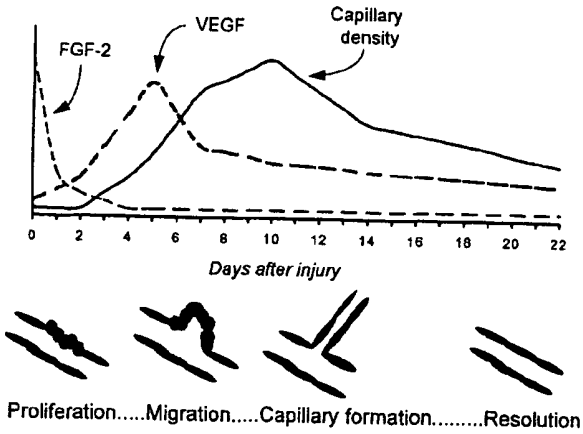

Figure 6. A model of angiogenesis in the healing wound. Immediately after injury, FGF-2 is quickly released, providing an early stimulus for endothelial cell proliferation. As FGF-2 levels decline, VEGF is produced. VEGF provides a sustained stimulus for endothelial cell migration and differentiation into new capillary tubes. Capillary density reaches a maximum shortly after peak VEGF levels are observed. Capillary regression then begins either because of a lack of continued stimulus or through the direct effect of antiangiogenic mediators. The indicated time period for each event (provided in days) is representative of the sequence observed in both human wounds and in murine models of tissue repair.

might therefore allow more specific regulation of vessel growth and regression during proliferative and maturation phases of wound repair. Our results support a model of wound angiogenesis in which early endothelial proliferation is supported by FGF-2 and later capillary growth and differentiation is directed by VEGF (Figure 6). Such a model has support in *in vitro* systems as well, such as the rat aorta model of angiogenesis.[34] Whether or not VEGF persists as the primary mediator throughout the remainder of the repair process remains to be determined.

Regulation of the VEGF-mediated response in wounds may take many forms. FGF-2 appears to have a direct role in the VEGF response based on observations that FGF-2 can up-regulate VEGF production[35] and that FGF-2 and VEGF act synergistically in stimulating angiogenesis in some models.[36,37] Several other growth factors such as platelet derived growth factor and transforming growth factor-β may also regulate VEGF production.[38,39] One interesting possible mediator of VEGF activity in wounds is local hypoxia. VEGF has a well-described hypoxia-inducible element that is thought to account for its increased production in ischemic retinopathy, myocardial ischemia, and in areas of tumor necrosis.[11,16] Extensive work by Hunt and others[40,41] has demonstrated that wounds are characteristically hypoxic during early phases of repair and that angiogenic activity in wounds varies inversely with wound oxygen levels. VEGF has been proposed as a mediator of this effect.[42,43] In theory, VEGF production and VEGF-mediated angiogenic activity would rise in the early hypoxic wound and then fall when neovascularization is complete and wound perfusion is restored.

While our results describe the initiation of a VEGF-mediated response, additional work is required to understand the regression of the vasculature that is seen in resolving wounds. Taken together, the elucidation of the mechanisms responsible for initiating and driving the angiogenic response in wounds might find broad application in areas such as the augmentation of wound repair, improving collateralization of ischemic limbs and myocardium, and understanding tumor growth.

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